Striatal molecular alterations in HD gene carriers: a systematic review and meta-analysis of PET studies

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Word count: 3,574

No of References: 78

Keywords

Manifest, premanifet, Huntington's disease gene carriers; PET.

ABSTRACT

BACKGROUND

Over the past years, PET imaging studies have investigated striatal molecular changes in premanifest and manifest Huntington's disease gene expansion carriers (HDGECs), but they have yielded inconsistent results.

OBJECTIVE

To systematically examine the evidence of striatal molecular alterations in manifest and premanifest HDGECs as measured by PET imaging studies.

METHODS

MEDLINE, ISI Web of Science, Cochrane Library and Scopus databases were searched for articles published until 7th June 2017 that included PET studies in manifest and premanifest HDGECs. Meta-analyses were conducted with random effect models and heterogeneity was addressed with I^2 index, controlling for publication bias and quality of study. The primary outcome was the standardized mean difference (SMD) of PET uptakes in the whole striatum, caudate, and putamen in manifest and premanifest HDGECs compared with healthy controls (HCs).

RESULTS

Twenty-four out of 63 PET studies in premanifest (n=158) and manifest (n=191) HDGECs and HCs (n=333) were included in the meta-analysis. Premanifest and manifest HDGECs showed significant decreases in dopamine D_2 receptors in caudate (SMD=−1.233, 95% CI=−1.753 to −0.713, P<0.0001; SMD=−5.792, 95% CI=−7.695 to −3.890, *P*<0.0001) and putamen (SMD=−1.479, 95% CI=−1.965 to −0.992, P<0.0001; SMD=−5.053, 95% CI=−6.558 to −3.549, *P*<0.0001), in glucose metabolism in caudate (SMD=−0.758, 95% CI=−1.139 to −0.376, P<0.0001;

SMD=−3.738, 95% CI=−4.880 to −2.597, *P*<0.0001) and putamen (SMD=−2.462, 95% CI=−4.208 to −0.717, P=0.006; SMD=−1.650, 95% CI=−2.842 to −0.458, *P*<0.001) and in striatal PDE10A binding (SMD=−1.663, 95% CI=−2.603 to −0.723, P=0.001; SMD=−2.445, 95% CI=−3.371 to −1.519, *P*<0.001).

CONCLUSIONS

PET imaging has the potential to detect striatal molecular changes even at the early premanifest stage of HD, which are relevant to the neuropathological mechanisms underlying the development of the disease.

INTRODUCTION

Huntington's disease (HD) is an inherited, neurodegenerative disorder caused by CAG repeat expansion in *huntingtin* gene (HTT). HD is clinically characterized by progressive motor dysfunction, cognitive decline, and psychiatric disturbances and will eventually lead to death, typically 15-20 years following symptomatic onset.[1] The onset of symptoms is inversely associated with the size of the CAG repeat expansion and most commonly occurs at the age of mid-40s.[1] However, subclinical changes and pathological processes are thought to precede the initiation of symptoms by several years.[2] The availability of genetic testing and the full penetrance of HTT mutation in people with more than 40 CAG expansions[3] provide a unique window of opportunity to examine the pattern of signs, symptoms, and neurobiological changes as they emerge, and study the clinical course of HD before the development of overt symptoms. There is an urgent need to identify biomarkers that are able to monitor disease progression and assess the development and efficacy of novel disease modifying drugs.

HD pathology is characterised by the formation of intranuclear inclusions of mutated huntingtin preferentially in the striatal GABAergic medium spiny neurons (MSNs). These aggregates hamper intracellular processes, such as gene transcription, protein trafficking, neurotransmitters release and mitochondrial function, leading to the loss of striatal MSNs.[1] Thus striatal molecular changes have great relevance to HD pathology and may provide a valuable tool to monitor disease progression and assess the efficacy of novel disease-modifying drugs.

Positron emission tomography (PET) is a molecular imaging technique for the quantitative and non-invasive imaging of biological functions. The distribution and kinetic profiles of compounds targeting specific biological molecules in tissue reflect specific biological functions in the living body. There are no good alternatives to PET in directly evaluating human neurochemistry. Previous PET imaging studies investigating striatal molecular changes in premanifest and manifest HDGECs have yielded inconsistent results mainly due to the heterogeneous and small sample size and different inclusion criteria used in these studies.[4]

In this systemic review and meta-analysis, we aim to systematically examine the evidence of *in vivo* striatal molecular changes in premanifest and manifest HD gene expansion carriers (HDGECs) as measured by PET imaging studies and to quantitatively estimate their magnitude. We hypothesize that striatal molecular changes are consistently impaired in manifest HDGECs as compared to controls as key neurobiological marker of the disease. These molecular changes may be already evident at the premanifest stage although the magnitude of these alterations may be more severe in the manifest as compared to premanifest HDGECs.

METHODS

The study was designed according to the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines and recommendations from the Cochrane Collaboration and Meta-analysis Of Observational Studies in Epidemiology (MOOSE).[5]

Search Strategy

MEDLINE, ISI Web of Science, Cochrane Library and Scopus databases electronic databases were searched for articles published from 1980 until 7th June 2017. Only full manuscripts were included in this meta-analysis. Gray literature (ie, abstract or conference proceedings) was not considered as a priority asset of our systematic review. Studies were identified, combining the following major Medical Subject Headings: "Huntington's disease" and "PET" combined with text and key words for MEDLINE as example: (("Huntington Disease" [MeSH Terms] OR "Huntington's" OR "Huntington's chorea" OR "chorea" [MeSH Terms] OR "hereditary chorea" OR "progressive chorea" OR "late onset Huntington disease" OR "juvenile Huntington disease" OR "akinetic rigid variant Huntington disease") AND ("Positron-Emission Tomography" [MeSH Terms] OR "positron emission tomography" OR "PET")). Additional eligible studies were identified through manual screening of the reference lists of studies included in our analysis. Corresponding authors were contacted by e-mail requesting meta-analytical details that were not included in the original manuscripts.

Selection Criteria

All selected titles and abstracts were independently reviewed by two authors (FN, GP) and then discussed with a third independent author (MP). Selected studies were eligible if they met the following criteria: (a) cross-sectional, case control or longitudinal studies including manifest or premanifest HDGECs compared with a healthy control (HC) group, (b) published in peer-reviewed international journals in English language, (c) confirmed HDGECs diagnosis on the basis of clinical symptoms and/or positive genetic test for CAG repeat, (d) classification as premanifest HDGECs as established by positive genetic test for CAG repeat and

absence of motor signs based on the standardized total motor score (TMS) subscale (TMS = 0) of the Unified Huntington Disease Rating Scale (UHDRS) with a diagnostic confidence level of 0,[6] and (e) PET measures in caudate, putamen or whole striatum reported as mean ± standard deviation (SD) in premanifest and manifest HDGECs and healthy control (HC) subjects. We excluded the following studies: (a) PET studies reporting changes in PET measures in subjects at risk of HD but not tested for CAG repeat (n=9)[7-15]; (b) studies using only Statistical Parametric Mapping (SPM) analyses (n=12)[16-27]; (c) studies including overlapping samples. In cases of 2 or more studies from the same centre, we checked for overlapping samples by contacting the authors to verify that there was not a significant overlap in the samples. Striatal PET radioligands of interest included: [^{11}C]Raclopride (dopamine D_2 receptors), [^{11}C]SCH23390 (dopamine D_1 receptors), [^{11}C]PK11195 (microglial activation), [^{18}F]FDG (glucose metabolism), [^{11}C]IMA107 [phosphodiesterase 10A (PDE10A)], [^{18}F]JNJ42259152 (PDE10A), [^{18}F]MNI-659 (PDE10A), [^{11}C]FMZ (GABA benzodiazepine receptor), [^{18}F]CPFPX [adenosine A_1 (A_{1A}) receptor], [^{11}C]β-CIT [dopamine transporter (DAT)], [^{11}C]DTBZ [vesicular monoamine transporter type-2 (VMAT2)] and [^{18}F]MK9470 [cannabinoid type 1 (CB_1) receptor].

Risk of bias in included studies

The quality of the included studies was assessed by Newcastle-Ottawa Scale (NOS).[28] NOS is characterized by eight items including selection, comparability, and exposure (case-control studies) or outcome (cohort studies). The scale ranged from zero to six stars, the highest degree representing the greatest methodological quality. Disagreement was resolved by consensus and by opinion of a third reviewer (MP).

The presence of publication bias was explored by performing the test for asymmetry of the funnel plot by Egger.[29]

Data extraction

Two reviewers (FN, GP) independently completed the data extraction. The recorded variables for each article included in the meta-analysis were study year, author first name, disease stage (premanifest, manifest), gender, mean age of participants, number of participants, type of radiotracer used, disease duration (years), CAG repeat, 5-year probability to symptom onset according to the Langbehn formula,[3] 90% probability to symptom onset according to revised survival analysis formula for determining time to symptom onset.[3]

Statistical Analysis

Data were analysed using Comprehensive Meta-Analysis software, version 2 (Biostat, Englewood, N.J.). PET uptake in manifest and premanifest HDGECs compared with HCs was estimated through the standardized mean difference (SMD). The mean difference in the primary outcome measures (PET uptake) between patients (premanifest and manifest HDGECs) and HC group was standardized by calculating the difference between the two mean changes (difference of patients and HCs score) divided by the pooled SD of the difference scores. A negative change of the standardized mean difference (SMD) indicates a larger reduction in our primary outcome measures in the patients (premanifest and manifest HDGECs) as compared to HCs. Independent meta-analyses across each type of radiotracer were carried out. The results were pooled using the inverse variance method. Heterogeneity was assessed using I^2 statistic that accounts of between-study (or inter-study) variability as

opposed to within-study (or intra-study) variability. Because of latent clinical heterogeneity, random effect models were used to synthesize data instead of fixed effect model, independently from statistical evidence of heterogeneity.[30] Heterogeneity was considered substantial if I^2 value was greater than 50%.[31] For completeness and clarity, we additionally calculated the percentage of change in the primary outcome measures between the HDGECs and HCs groups. All reported test results were two-tailed and statistical significance was set to a $P<0.05$.

Sensitivity analyses

To investigate the influence of individual studies on the meta-analytical results, we undertook one study-removed analysis by omitting one study in each meta-analysis and recalculating the pooled estimates on remaining studies.[32] Meta-regression analysis to explore the influence of potential effect modifiers on striatal changes was not performed due to the small number of PET studies per each target (less than 10 studies).[33]

RESULTS

Meta-analytical database

The combined search strategies yielded a total of 702 references identified, of which 63 were retrieved for detailed full-text evaluation and 24 were finally included in the meta-analysis (Figure 1).[34-59] PET studies included in the systematic review and quantitative meta-analysis investigated striatal changes in dopamine D_1[39,41,44] and D_2 receptors,[39-42,44,46,47,51] glucose metabolism,[34-37,40,44,45,49,50,54] microglial activation,[46,59] A_{1A} receptor,[52] GABA benzodiazepine receptor,[38] and presynaptic molecular changes (DAT and VMAT2)[42,43] in manifest and premanifest HDGECs compared with HCs.

PET studies investigating the expression of PDE10A in premanifest and manifest HDGECs[53,55,56] and CB_1 receptor density in manifest HDGECs[48] reported PET molecular changes in the whole striatum and were not included in the pooled analysis. Four PET studies have reported significant increases in striatal microglial activation in manifest HDGECs[46,58-60] but due to overlapping cohort of subjects we have included only one study in the meta-analysis.[46] Characteristics of the studies included are summarised in Table 1 and 2. The study populations in this meta-analysis included 333 HCs (mean age=46.9 years; 58.9 % male), 158 premanifest HDGECs (mean age =39 years; 40.6% male; mean CAGr=43.1) and 191 manifest HDGECs (mean age =47.7years; 54.2% male; mean CAGr =43.5). Manifest HDGECs had mean disease duration of 4.29 years (range 2.2 to 7.25) and premanifest HDGECs were on average 19.2 years (range 10.3 to 25) before the predicted symptomatic onset (90% probability). The quality of included studies was moderate or good, varying from three to six NOS stars (Table S1).

Premanifest HDGECs

Premanifest HDGECs showed significant decreases in dopamine D_2 receptors in caudate (SMD=−1.233, 95% CI=−1.753 to −0.713, P<0.0001; I^2=25.7%) and putamen (SMD=−1.479, 95% CI=−1.965 to −0.992, P<0.0001; I^2=10.1%; Figure 2), in glucose metabolism in caudate (SMD=−0.758, 95% CI=−1.139 to −0.376, P<0.0001; I^2=0.0%) and putamen (SMD=−2.462, 95% CI=−4.208 to −0.717, P=0.006; I^2=88.6%; Figure 2) and in striatal PDE10A binding (SMD=−1.663, 95% CI=−2.603 to −0.723, P=0.001; I^2=24.0%; Figure 3A). Significant increases in microglial activation were observed in caudate (SMD=1.491, 95% CI=0.586 to 2.395,

P=0.001; I^2=0.0%) and putamen (SMD=1.355, 95% CI=0.467 to 2.242, *P*=0.003; I^2=0.0%) of premanifest HDGCs (Figure 2).

One PET study has assessed changes in striatal A_{1A} receptor levels in premanifest HDGECs.[52] No significant differences were found in A_{1A} receptor levels in caudate (SMD=0.500, 95% CI=−0.141 to 1.142, *P*=0.126) and putamen (SMD=0.250, 95% CI=−0.386 to 0.886, *P*=0.441) of premanifest HDGECs compared with the group of HCs (Figure 2).

Manifest HDGECs

Manifest HDGECs showed significant decreases in dopamine D_2 receptors in caudate (SMD=−5.792, 95% CI=−7.695 to −3.890, *P*<0.0001; I^2=76.9%) and putamen (SMD=−5.053, 95% CI=−6.558 to −3.549, *P*<0.0001; I^2=69.3%), D_1 receptors in caudate (SMD=−3.648, 95% CI=−5.333 to −1.964, *P*<0.001; I^2=58.1%) and putamen (SMD=−4.628, 95% CI=−8.027 to −1.230, *P*=0.008; I^2=86.0%) and glucose metabolism in caudate (SMD=−3.738, 95% CI=−4.880 to −2.597, *P*<0.0001; I^2=71.4%) and putamen (SMD=−1.650, 95% CI=−2.842 to −0.458, *P*<0.001; I^2=86.4%; Figure 4).

Significant decreases in striatal PDE10A (SMD=−2.445, 95% CI=−3.371 to −1.519, *P*<0.001; I^2=0.0%) and CB_1 receptor levels (SMD=−0.758, 95% CI=−1.472 to −0.044, *P*=0.037) were also observed in manifest HDGECs compared to the group of healthy controls (Figure 3B). Increases in microglial activation were observed in the caudate (SMD=1.748, 95% CI=0.690 to 2.806, *P*=0.001) and putamen (SMD=1.784, 95% CI=0.719 to 2.848, *P*=0.001) of manifest HDGECs (Figure 4).

Manifest HDGECs showed modest decreases in A_{1A} receptor levels in caudate (SMD=−0.950, 95% CI=−1.741 to −0.159, *P*=0.019) and putamen (SMD=−0.855,

95% CI=−1.642 to −0.069, *P*=0.033; Figure 4). Significant decreases in GABA benzodiazepine receptors were found in the caudate (SMD=−1.612, 95% CI=−2.915 to −0.310, *P*=0.015) but not in the putamen of manifest HDGECs (SMD=−0.417, 95% CI=−1.560 to 0.727, *P*=0.475; Figure 4).

Changes in presynaptic dopamine terminals were observed in two PET studies.[42,43] Decreases in DAT levels were found in caudate (SMD=−3.007, 95% CI=−4.817 to −1.198, *P*=0.001) and putamen (SMD=−3.110, 95% CI=−4.953 to −1.268, *P*=0.001) in manifest HDGECs compared to the group of HCs. $VMAT_2$ levels were significantly decreased in the putamen (SMD=−0.550, 95% CI=−0.667 to −0.433, *P*<0.0001) and increased in the caudate (SMD=1.304, 95% CI=0.755 to 1.854, *P*<0.0001) of manifest HDGECs (Figure 4).

Publication Bias and Sensitivity Analysis

The Egger test was significant for [^{18}F]FDG uptake in the caudate (*P*=0.022) and putamen (*P*=0.018) of manifest HDGECs indicating a risk of publication bias for this radioligand. Egger tests for the other outcome measures were not significant. Robustness of meta-analytic findings was confirmed by sequentially removing each study and re-analyzing the remaining data set (producing a new analysis for each study removed). The results remained essentially unchanged in direction and magnitude (results are available from the authors upon request).

Supplementary analyses

The supplementary analysis (Table 3) showed 24-25.5% and 59-60% decreases in caudate and putamen dopamine D_2 receptor levels in premanifest and manifest HDGECs, respectively, and 55.7-57.4% decreases were seen in caudate and putamen

dopamine D_1 receptor binding in manifest HDGECs. Glucose hypometabolism ranged between 6-11.2% and 41.4-51.3% decreases in the caudate and putamen of premanifest and manifest HDGECs, respectively. Premanifest HDGECs showed increases in microglial activation by 63.7% in caudate and 43.7% in putamen. Striatal PDE10A levels were decreased by 24.6% in premanifest and by 61.8% in manifest HDGECs compared to the HC group.

DISCUSSION

This is a comprehensive meta-analysis investigating *in vivo* striatal molecular changes in premanifest and manifest HDGECs. We found that PET molecular imaging has the potential to detect striatal molecular changes even at the early premanifest stage of HD, which are relevant to the neuropathological mechanisms underlying the development of the disease. Striatal molecular changes were more severe in manifest as compared to premanifest HDGECs.

Manifest HDGECs showed significant decreases in dopamine D_1, D_2 receptor binding and glucose metabolism in caudate and putamen compared to HCs. Moreover, striatal PDE10A expression and CB_1 receptor levels were decreased in manifest HDGECs whereas increased microglial activation was found in the caudate and putamen of manifest HDGECs. The greatest differences were observed in dopamine D_1 (caudate SMD=−3.648; -57.7%, putamen SMD=−1.650; -55.7%) and D_2 (caudate SMD=−5.792; -59%; putamen SMD=−5.053; -60%) receptor binding and striatal PDE10A expression (striatal PDE10A SMD=−2.445; -61.8%). Our findings are in line with the known pathological feature of HD affecting preferentially striatal GABAergic MSNs expressing dopamine receptors.[61] Greater decreases were observed

in dopamine D_2 receptor binding compared to dopamine D_1 binding, in line with previous postmortem studies indicating preferential degeneration of dopamine D_2 striatopallidal external projection neurons in HD.[62] Previous PET studies have shown that decreases in dopamine receptors are associated with longer disease duration and symptom severity highlighting the importance of dopaminergic signalling as a marker for monitoring disease progression.[41,42] In premanifest HDGECs, dopamine D_2 receptor binding was also significantly decreased in caudate (SMD=−1.233; -24%) and putamen (SMD=−1.479; -25.5%) compared to the HCs suggesting that loss of dopamine D_2 receptor binding can occur at the early stages of the disease. In premanifest HDGECs, the magnitude of striatal changes in dopamine D_2 receptor binding was half of those observed in manifest HDGECs. In summary, the magnitude of the decrease in D_1 binding in manifest HDGECs was similar to that of D_2 binding, whereas only D_2 binding was significantly decreased in premanifest HDGECs. This might presumably reflect preferential involvement of the indirect pathways in early stage of the disease, with less selective involvement as disease progresses, but these differences are unlikely to be apparent with disease progression.

Striatal PDE10A expression was also severely reduced in manifest HDGECs and in premanifest HDGECs, though to a lesser degree compared to manifest HDGECs (striatal SMD=−1.663; -24.6%). Preclinical studies have suggested an important role of PDE10A in HD pathology.[63-65] Mutant HTT decreases PDE10A mRNA expression levels in the striatum[63,66] and inhibition of PDE10A reduces the loss of striatal and cortical neurons and delays the development of neurological deficits in HD animal models.[64,65] A recent preclinical study has shown that chronic PDE10 inhibition starting at presymptomatic ages decreases the onset of mHTT-induced corticostriatal

transmission deficits and improves cortically driven indirect pathway activity in HD animal models.[67] Our results confirm the relevance of this enzyme in HD pathology and suggest that PDE10A could be a potential novel biomarker of striatal MSNs integrity. However, due to the small sample size and number of studies, we were unable to directly compare loss of dopamine receptor binding and PDE10A decreases. A recent PET study has investigated longitudinal PDE10A changes in a small cohort of two premanifest and six manifest HDGECs.[68] The mean annualised rate of decline in PDE10A was 16.6% in caudate and 6.9% in putamen of HDGECs. The rate of annual change of PDE10A expression was greater than the one observed in dopamine D_2 receptors highlighting the role of this enzyme in HD pathology.[40,69,70] There is currently one ongoing study, PEARL-HD, evaluating the expression of PDE10A enzyme and dopamine D_2 receptor levels using [^{18}F]MNI-659 and [^{11}C]raclopride in premanifest and manifest HDGECs and HCs.[71] In this study, [^{11}C]raclopride and [^{18}F]MNI-659 binding were significantly lower in HDGECs compared with HCs. In manifest HDGECs stage I dopamine D_2 receptors and PDE10A availability were decreased by 63% and 91% in the caudate, and by 43% and 69% in the putamen compared to HCs. In premanifest HDGECs, the corresponding reductions were 32% and 53% in the caudate, 31% and 43% in the putamen. These preliminary results show that striatal PDE10A is already more severely reduced than striatal D_2 receptors in HD, even at the earliest stages of the disease.[71]

Striatal CB_1 receptor levels were decreased in manifest HDGECs (striatal SMD=−0.758). CB_1 receptors are mainly expressed on GABAergic striatal MSNs and are a key modulator of synaptic transmission in the brain,[72] thus they may play an important role in the pathogenesis of HD. Further studies investigating CB_1 receptor

levels in premanifest HDGECs and using different CB1 PET radioligand with higher brain uptake, faster kinetics, better time stability, and robust measurements of distribution volume are needed in order to further elucidate the role of these receptors in the pathophysiology of HD.

Glucose metabolism decreases observed in this meta-analysis were smaller compared to loss of PDE10A and dopamine D_2 receptor binding in both premanifest (caudate SMD=−0.758; -6%; putamen SMD=−2.462; -11.2%) and manifest (caudate SMD=−3.738; -51.3%; putamen SMD=−1.650; -41.4%) HDGECs compared to the group of HCs. These findings may suggest that glucose metabolism is a less sensitive marker of striatal dysfunction at the early stages of the disease. Greater reductions in glucose metabolism were observed in caudate than in putamen of manifest and premanifest HDGECs. This is consistent with histological and MRI studies showing that HD-related striatal atrophy follows a topographical dorsoventral and caudorostral gradient affecting earlier the tail and body of the caudate.[73] Previous PET studies have found a significant association between decreased caudate glucose metabolism and cognitive decline in manifest HD patients.[36,37] We were unable to investigate the potential effect of cognitive impairment modifiers on caudate glucose metabolism due to the lack of cognitive measures. One limitation in the interpretation of glucose metabolism analysis is the different methods used to quantify [^{18}F]FDG uptake (i.e. glucose absolute values, normalised to cortical or cerebellar or global metabolic values), that has been taken in account using subgroups analysis. Additional limitation is the presence of publication bias for both caudate and putamen glucose uptake; caution should be taken when considering the importance of altered striatal glucose metabolism in HDGECs. Although glucose metabolism deficits are an important

component of HD pathogenesis,[74] [^{18}F]FDG PET acquisition has limitations and heavily depends on the conditions of the study. For instance, blood glucose level may influence the image quality.[75] High intracellular glucose and circulating insulin levels increase [^{18}F]FDG uptake by the muscle and further reduce the uptake in the brain. Thus, [^{18}F]FDG PET of the brain is affected both qualitatively and quantitatively by hyperglycemia.[76] It has been recently suggested that diabetes and poor glycemic control decrease [^{18}F]FDG uptake in cortical areas associated with Alzheimer's disease, whereas does not influence the accumulation of amyloid-β related tracer [^{11}C]PiB.[77] Moreover, several psychotropic drugs including benzodiazepine can decrease the global brain activity and affect brain glucose metabolism.[78] Lastly, sensorial input may also cause a bias since they can alter regional glucose metabolism.

We found increased microglial activation in caudate and putamen of premanifest (caudate SMD=1.491; +43.6%; putamen SMD=1.355; +63.7%) and manifest (caudate SMD=1.748; putamen SMD=1.784) HDGECs. Microglial activation could contribute to the HD neurodegenerative processes.[79] Microglia expressing mutant huntingtin become over-activated in response to stimulation[80] and promotes the expression of increased pro-inflammatory cytokines contributing to tissue damage and pathogenesis of HD.[79] Previous PET studies have reported 50% increases in striatal microglial activation in manifest HDGECs[46,60] that correlated with loss of striatal dopamine D_2 receptor binding and motor symptom severity.[60] In premanifest HDGECs, striatal microglial activation was also found increased and correlated with subclinical striatal neuronal loss of dopamine D_2 receptor binding and with higher probability of symptomatic onset over the next 5 years.[46,58,59] However, our results should be

interpreted cautiously due to the small sample size, limited number of studies included and radioligand limitation. [^{11}C]PK11195 shows high level of non-specific binding and a poor signal-to-noise ratio,[81] which complicates its quantification; moreover, test–retest data in control subjects showed only moderate intra-individual reproducibility[82] as compared to [^{11}C]raclopride.[83]

Other striatal molecular changes were observed in this meta-analysis. Striatal A_{1A} receptor levels were found decreased in manifest but not in premanifest HDGECs. Decreases in GABA benzodiazepine receptors were observed only in the caudate of manifest HDGECs. Striatal DAT binding was decreased in manifest HDGECs whereas decreases in $VMAT_2$ levels was found only in the putamen of manifest HDGECs. The increased VMAT2 binding observed in the caudate of manifest HDGECs might reflect loss of volume with increased density of nerve terminals projecting to the caudate, however the small sample size and difficulty in correcting for atrophy make this assumption speculative. Only one study reported decreases in both caudate and putamen DAT binding in manifest HDGECs. This study has several limitations including the small sample size (only five HDGECs), the lack of correction for striatal atrophy and the PET analysis method employed. Striatal [^{11}C]β-CIT uptake kinetics is not irreversible and do not fully satisfy the constraints of multiple-time graphical analysis (MTGA) model assumptions. Additionally, neuroleptics drugs and tetrabenazine, commonly used in HD, interfere with the release of dopamine at the presynaptic terminal. Both studies did not report whether HDGECs were on dopamine modulating drugs, which could have influenced VMAT2 and DAT availability. Therefore, these results should be interpreted cautiously and further studies in larger cohort of HDGECs using appropriate partial volume

correction methods are needed in order to further elucidate the integrity of presynaptic dopaminergic terminals in HD.

The main limitation of our meta-analysis is that it was carried out on a few studies and this limited meta-regression analysis. Additional limitations include the small sample size and a low quality of some studies that represent a potential risk of bias. Several PET studies included in this meta-analysis did not employ partial volume correction methods,[34-37,38-42,45,47,50,51,54] thus striatal neuronal loss occurring in HD might have influenced the outcome measurement accuracy in relation to the actual target change.

CONCLUSIONS

It is challenging to perform PET imaging studies in HDGECs due to low prevalence and progressive course of the disease leading to severe cognitive and motor deficits. This systemic review and meta-analysis is nevertheless the best evidence to date demonstrating significant striatal molecular changes in manifest and to a lesser degree in premanifest HDGECs. Despite 20 years of PET research in HDGECs, conclusions are limited and further larger studies are needed for understanding the biological signature of the different PET biomarkers across the stages of HDGECs, which could be used to monitor disease progression and response to medications in therapeutic trials. A longitudinal PET study that attempts to address multiple PET biomarkers across the different stage of HD will be able to characterize potential longitudinal progression and pharmacodynamic biomarkers that could be used as markers of treatment response in therapeutic development for HDGECs.

ACKNOWLEDGEMENTS

Marios Politis research is supported by Parkinson's UK, Edmond J. Safra Foundation, Michael J Fox Foundation (MJFF), and NIHR BRC. Gennaro Pagano research is supported by Edmond J. Safra Foundation.

Authorship

M.P. and C.S. conceptualised the study. F.N. and G.P. collected the studies, extracted the data, and made the statistical analysis. F.N. wrote the first draft of the paper. P.F.P., M.P., A.W. L.M., and C.S. validated the extracted data, and contributed to the analysis interpretation and to writing the paper.

Potential Conflicts of Interest

The authors report no conflict of interest.

Study Funding

None

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FIGURE LEGENDS

Fig. 1 Search strategy for identifying PET studies in manifest and premanifest HDGECs.

Fig. 2 Pooled analysis (SMD) for *in vivo* PET molecular changes in the caudate and putamen of premanifest HDGECs.

Fig. 3 Pooled analysis (SMD) for *in vivo* striatal PET molecular changes in premanifest (A) and manifest (B) HDGECs.

Fig. 4 Pooled analysis (SMD) for *in vivo* PET molecular changes in the caudate and putamen of manifest HDGECs.

Table 1 PET studies included in the meta-analysis for the group of premanifest Huntington's disease gene expansion carriers

Study and Group	**Radioligand**	**N**		**Age (years)**		**Prob to onset**		**CAGr**	
		Total	**Male (%)**	**Mean**	**Range**	**p5 years[1]**	**90% p to onset[2]**	**Mean**	**Range**
D2 receptor									
Antonini et al., 1996	[^{11}C]raclopride								
HC		14	-	30	21-49				
premanifest HD		10	50	30	21-41	0.12	26.7	43.9	40-47
Politis et al., 2008	[^{11}C]raclopride								
HC		9	89.5	41.2	5.5*				
premanifest HD		10	50	41.9	32-61	0.33	16.9	43.4	39-48
van Oostrom et al., 2009	[^{11}C]raclopride								

HC		11	54	48.4	30-65				
premanifest HD		18	44	39.9	29-56	0.21	19.5	43	39-47
Tang et al., 2013	[^{11}C]raclopride								
HC		12	42.5	50.1	15.6*				
premanifest HD		12	46.8	48.3	11.0*	0.33	10.3	41.6	1.7*
Microglial activation									
Politis et al., 2008	[^{11}C]PK11195								
HC		10	89.5	56.7	11.7*				
premanifest HD		10	50	41.9	32-61	0.33	16.9	43.4	39-48
Politis et al., 2015	[^{11}C]PK11195								
HC		12	58.3	39.4	28-65				
premanifest HD		12	41.7	41.1	29-59	0.32	17.9	43.7	40-48
Glucose metabolism									
Antonini et al., 1996	[^{18}F]FDG								

HC		20	-	34	22-44				
premanifest HD		13	50	30	21-41	0.17	23.7	43.9	40-47
Feigin et al., 2007	[^{18}F]FDG								
HC		12	42.5	50.1	15.6*				
premanifest HD		12	46.8	48.3	11.0*	0.33	10.3	41.6	1.7*
Ciarmiello et al., 2012	[^{18}F]FDG								
HC		21	57.1	68.1	48-91				
premanifest HD		43	55.8	37.3	19-59	0.25	18	43.8	39-54
Herben-Dekker et al., 2014	[^{18}F]FDG								
HC		11	33	42.5	26-54				
premanifest HD		22	36	38.7	31-56	0.19	22.4	42.6	39-47
Phosphodiesterase 10 A									
Russell et al., 2014	[^{18}F]MNI-659								

HC		9	55	46.1	29-71				
premanifest HD		3	0	32.4	32-34	0.18	22.3	44.3	42-47
Niccolini et al., 2015	[^{11}C]IMA107								
HC		12	66.7	40	28-50				
premanifest HD		12	58.3	41.1	32-52	0.16	25	41.8	40-44
Adenosine A1 receptor									
Matush et al., 2014	[^{18}F]CPFPX								
HC		36	63.9	49.6	16*				
premanifest HD		13	23.1	39.1	7*	0.19	13	42.8	1.4*

[1]p5 years= 5-year probability to symptoms onset according to the Langbehn formula;[10] [2]90% p to onset = predicted years to Huntington's disease symptoms onset (90% probability) calculated on the basis of the variant of the survival analysis formula described by Langbehn;[5] HC=healthy controls; HD=Huntington's disease; *Standard deviation.

Table 2 PET studies included in the meta-analysis for the group of manifest Huntington's disease gene expansion carriers

Study and Group	PET ligand	N		Age (years)		Disease duration (years)	CAGr	
		Total	Male (%)	Mean	Range		Mean	Range
D1 receptor								
Turjanski et al., 1995	[^{11}C]SCH23390							
HC		6	-	51	31-78			
Manifest HD		10	50	48	29-72	3		
Backman et al., 1997	[^{11}C]SCH23390							
HC		5	60	48	7.8*			
Manifest HD		5	60	49.4	7.6*	5.6		
Furtado et al., 2005	[^{11}C]SCH23390							
HC		4	-	-	-			
Manifest HD		7	-	-	-	-		

D2 receptor								
Turjanski et al., 1995	[^{11}C]raclopride							
HC		9	-	50	24-74			
Manifest HD		10	50	48	29-72	3		
Antonini et al., 1996	[^{11}C]raclopride							
HC		14	-	30	21-49			
Manifest HD		8	75	44	34-52	2.2	44	42-48
Ginovart et al., 1997	[^{11}C]raclopride							
HC		5	-	-				
Manifest HD		5	60	49.4	37-56	-	-	-
Backman et al., 1997	[^{11}C]raclopride							
HC		5	60	48	7.8*			
Manifest HD		5	60	49.4	7.6*	5.6	-	-
Politis et al., 2008	[^{11}C]raclopride							

HC		9	100	41.2	5.5*			
Manifest HD		9	44.4	46.8	39-54	7.25	41.5	36-51
Furtado et al., 2005	[^{11}C]raclopride							
HC		10	-	-	-			
Manifest HD		7	-	-	-	-	-	-
Microglial activation								
Politis et al., 2008	[^{11}C]PK11195							
HC		10	89.5	56.7	11.7*			
Manifest HD		9	44.4	46.8	39-54	7.25	41.5	36-51
Glucose metabolism								
Hayden et al., 1986	[^{18}F]FDG							
HC		7	-	49.3	23-66			
Manifest HD		10	50	45.3	33-61	2.6	-	-
Young et al., 1986	[^{18}F]FDG							

HC		10		37.5	25-65			
Manifest HD		10		40.5	25-65	-	-	-
Berent et al., 1988	[^{18}F]FDG							
HC		14	-	37.5	25-65	-		
Manifest HD		15	-	40.5	25-60	-	-	-
Kuwert et al., 1990	[^{18}F]FDG							
HC		20	71.4	41.1	25-65			
Manifest HD		23	52.2	42.7	25-65	-	-	-
Antonini et al., 1996	[^{18}F]FDG							
HC		20		34	22-44			
Manifest HD		8	75	44	34-52	-	44	42-48
Furtado et al., 2005	[^{18}F]FDG							
HC		10	-	-	-			
Manifest HD		7	-	-	-	-	-	-

Shin et al., 2013	[^{18}F]FDG							
HC		11	45.5	48.6				
Manifest HD		13	38.5	46.4	36-76	6.5	44.7	36-55
Phosphodiesterase 10 A								
Ahmad et al., 2014	[^{18}F]JNJ422591 52							
HC		11	63.6	56.8	47-78			
Manifest HD		5	80	50.8	42-70	3.2	44	43-46
Russell et al., 2014	[^{18}F]MNI-659							
HC		9	55	46.1	29-71			
Manifest HD		8	25	51.8	20-67	-	45.3	40-68
GABA benzodiazepine receptor								
Holthoff et al., 1993	[^{11}C]FMZ							
HC		6	-	50	13*			

Manifest HD		6	50	53	9*	4.5	-	-
Dopamine transporter								
Ginovart et al., 1997	[^{11}C]β-CIT							
HC		5	-	-				
Manifest HD		5	60	49.4	37-56	-	-	-
Vesicular monoamine transporter type-2								
Bohnen et al., 2000	[^{11}C]DTBZ							
HC		64	50	50	23-70			
Manifest HD		19	68.4	48	16*	-	-	-
Cannabinoid type 1 receptor								
van Laere et al., 2010	[^{18}F]MK9470							
HC		14	42.8	54.3	31-68			
Manifest HD		20	40	53.3	32-83	6	43.6	39-50
Adenosine A_1 receptor								

Matush et al., 2014	[^{18}F]CPFPX							
HC		36	63.9	49.6	16*			
Manifest HD		8	62.5	46.6	5*	2.3	43.3	1.5*

HC=healthy controls; HD=Huntington's disease; *Standard deviation

Table 3. Percentage change for the outcome measures between premanifest and manifest HDGECs and HCs.

Premanifest HDGECs									
	ROIs	Groups	Mean	S.D.	Min	25%th quartile	Median	75%th quartile	Max
D2	Caudate	pHDGECs	1.78	0.56	1.25	1.27	1.74	2.31	2.37
		HCs	2.33	0.67	1.49	1.64	2.47	2.88	2.89
		Differences	-0.24	-0.37	-0.73	-0.57	-0.52	-0.56	-0.11

		(% changes)	(-23.8%)						
	Putamen	pHDGECs	2.06	0.46	1.37	1.59	2.27	2.32	2.32
		HCs	2.76	0.51	2.07	2.24	2.86	3.19	3.26
		Differences (% changes)	-0.70 (-25.5%)	-0.05	-0.70	-0.65	-0.59	-0.87	-0.94
FDG	Caudate	pHDGECs	1.04	0.21	0.80	0.80	1.12	1.19	1.19
		HCs	1.10	0.20	0.88	0.88	1.18	1.25	1.25
		Differences (% changes)	-0.08 (-6.0%)	-0.08	-0.06	-0.06	-0.06	-0.07	0.01
	Putamen	pHDGECs	1.00	0.23	0.74	0.74	1.10	1.17	1.17
		HCs	1.13	0.18	0.93	0.93	1.20	1.26	1.26
		Differences (% changes)	-0.13 (-11.2%)	0.05	-0.19	-0.19	-0.10	-0.09	-0.09
PK	Caudate	pHDGECs	0.19	0.06	0.15	0.15	0.19	0.23	0.23

		HCs	0.07	0.09	0.01	0.01	0.07	0.13	0.13
		Differences (% changes)	+0.12 (+63.7%)	0.14	0.12	0.10	0.10	0.12	-0.03
	Putamen	pHDGECs	0.28	0.06	0.23	0.23	0.28	0.32	0.32
		HCs	0.16	0.04	0.13	0.13	0.16	0.18	0.18
		Differences (% changes)	+0.12 (+43.6%)	0.03	0.10	0.10	0.12	0.14	0.14
PDE10A	Striatum	pHDGECs	1.26	1.26	1.74	2.21	2.21	1.74	0.67
		HCs	1.81	1.81	2.30	2.79	2.79	2.30	0.69
		Differences (% changes)	-0.55 (-24.6%)	-0.55	-0.57	-0.58	-0.58	-0.57	-0.02
Manifest HDGECs									
	ROIs	Groups	Mean	SD	Min	25%th quartile	Median	75%th quartile	Max

D1	Caudate	HDGECs	0.55	0.10	0.47	0.47	0.52	0.67	0.67
		HCs	1.3	0.15	1.13	1.13	1.38	1.39	1.39
		Differences (% changes)	-0.75 (-57.4%)	-0.04	-0.66	-0.66	-0.86	-0.72	-0.72
	Putamen	HDGECs	0.59	0.23	0.39	0.39	0.53	0.84	0.84
		HCs	1.32	0.04	1.28	1.28	1.34	1.35	1.35
		Differences (% changes)	-0.74 (-55.7%)	0.19	-0.89	-0.89	-0.81	-0.51	-0.51
D2	Caudate	HDGECs	0.99	0.43	0.22	0.69	1.10	1.28	1.45
		HCs	2.41	0.37	2.08	2.08	2.29	2.86	2.89
		Differences (% changes)	-1.42 (-59.0%)	0.06	-1.85	-1.39	-1.19	-1.58	-1.44
	Putamen	HDGECs	1.03	0.56	0.15	0.58	1.15	1.49	1.53
		HCs	2.58	0.37	2.25	2.32	2.46	2.89	3.26

		Differences (% changes)	-1.55 (-60.0%)	0.18	-2.07	-1.73	-1.30	-1.38	-1.73
FDG	Caudate	HDGECs	2.31	1.65	0.47	0.66	2.43	3.35	5
		HCs	13.16	20.82	0.91	1.11	6.87	19.73	55.2
		Differences (% changes)	-1.8 (-51.3%)	-19.17	-0.44	-0.448	-4.445	-16.38	-50.2
	Putamen	HDGECs	3.09	2.13	0.59	0.81	3.25	4.89	6.1
		HCs	5.28	3.25	1.05	1.21	6.73	7.82	8.2
		Differences (% changes)	-2.19 (-41.4%)	-1.13	-0.46	-0.40	-3.48	-2.93	-2.1
PDE10A	Striatum	HDGECs	1.33	0.25	1.15	1.15	1.33	1.50	1.50
		HCs	3.47	0.96	2.79	2.79	3.47	4.15	4.15
		Differences (% changes)	-1.64 (-61.8%)	-1.64	-2.15	-2.65	-2.65	-2.15	-0.71

Mean, standard deviation, minimum, 25th, 50th and 75th quartile and maximum are presented for each group separately and all studies together. The % change is shown for premanifest and manifest HDGECs and HC groups separately as well as the difference in % change between HDGECs and HCs groups.